Integrated Biology Solutions

PROTEOMICS GENOMICS INFORMATICS

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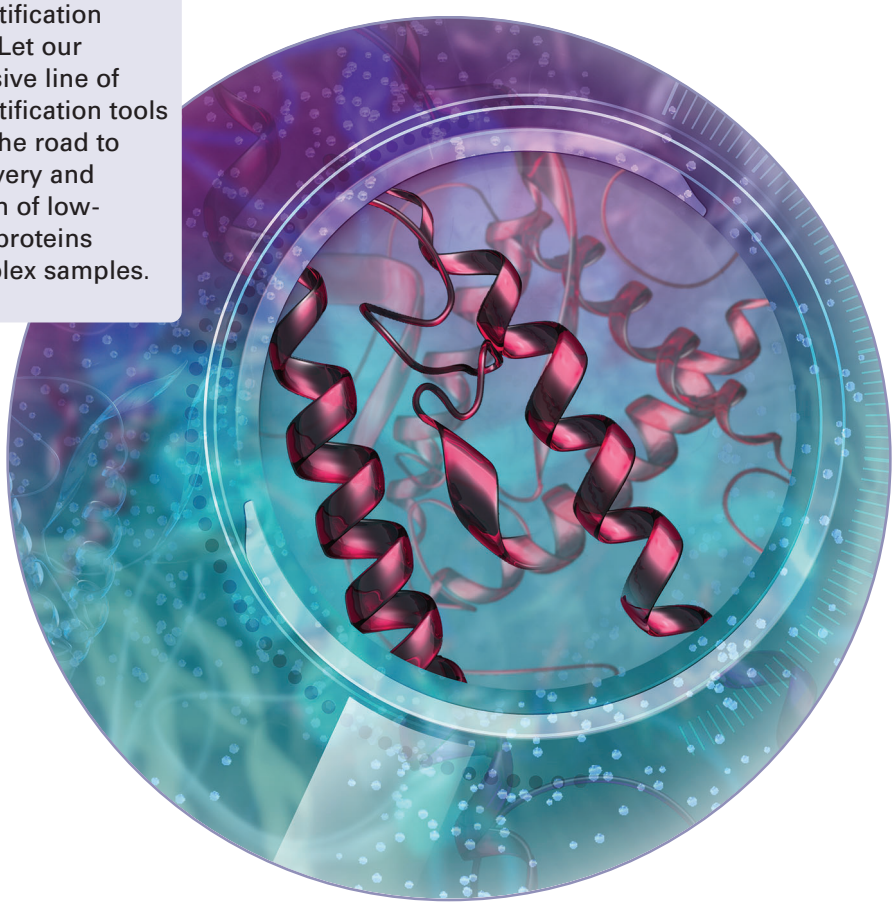
AT A GLANCE

Agilent Technologies has developed and optimized products and solutions to address an entire range of protein identification challenges. Let our comprehensive line of protein identification tools put you on the road to faster discovery and identification of low-abundance proteins within complex samples.



LC/MS solutions to discover and identify more proteins from complex samples

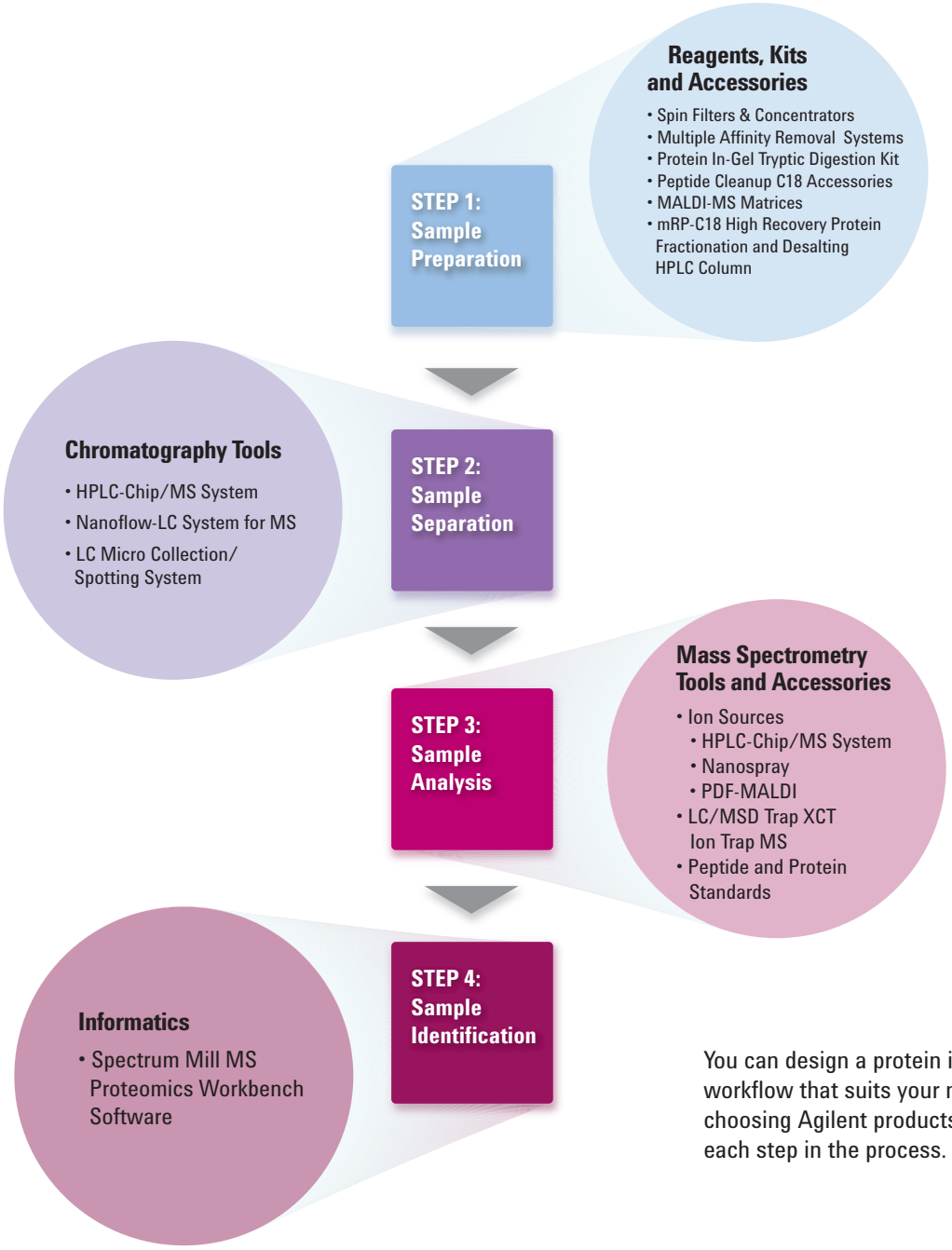
Agilent Technologies

A comprehensive and integrated workflow for fast and accurate protein identification

The identification of proteins from biological samples presents unique challenges. A single sample can contain thousands of proteins whose abundances vary over many orders of magnitude. Often, the proteins of most interest are present at lower abundance.

Agilent Technologies offers an integrated suite of powerful tools that enables you to successfully prepare, separate, analyze, and identify your challenging protein samples.

Optimized preparation products, outstanding LC separation technologies, exceptionally sensitive ion trap mass spectrometer (MS) performance, and highly powerful software all combine to deliver unparalleled analysis of complex proteomic samples.



You can design a protein identification workflow that suits your needs by choosing Agilent products to address each step in the process.

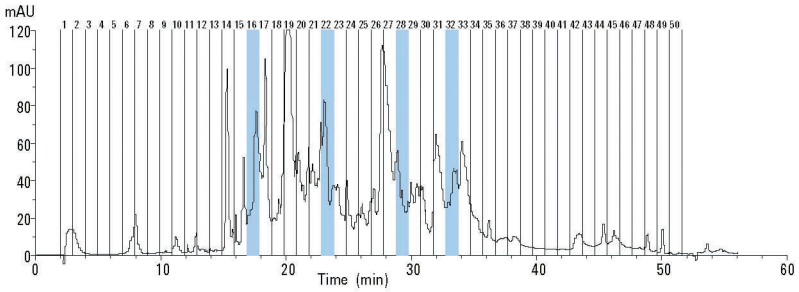
Sample preparation technologies that optimize analytical performance

By combining our proprietary reagents and consumables with our instrument systems, you can take advantage of enhanced performance, reduced time-to-results, and an improved, easier workflow.

We have solutions for all stages of your sample preparation—from the removal of interfering, high-abundance serum proteins to in-gel tryptic digestion kits for effective and reproducible results.

Agilent's efficient sample preparation technologies improve the consistency and reliability of your results. Our expanding line of sample preparation solutions includes:

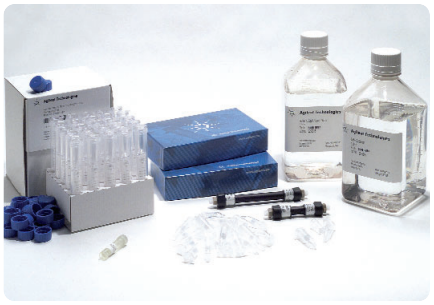
- **Multiple Affinity Removal systems** selectively immunodeplete multiple high-abundance serum proteins, enabling you to study low-abundance proteins that may act as prognostic or therapeutic disease biomarkers.
- **Macroporous Reversed-Phase C18 (mRP-C18) High-Recovery Protein Fractionation and Desalting HPLC column** recovers >98% of proteins and peptides from immunodepleted human, mouse, and rat serum samples. The number of identified proteins in the sample can increase as much as 10-fold when coupled with Agilent LC/MS systems.
- **Protein In-gel Tryptic Digestion kits** ensure effective and reproducible sample digestion, reduction, and alkylation with a simple protocol.
- **Peptide Cleanup C18 spin tubes and pipette tips** clean and desalt both large and small samples prior to downstream analysis.



Sample	Number of Proteins Identified
Human Serum*	40
Immunodepleted Human Serum**	181
Fraction 16	71
Fraction 22	97
Fraction 28	99
Fraction 32	181
Combined Fractions (16, 22, 28, 32)	394

Immunodepleted human serum in 50 separate fractions. After fractionation with the mRP-C18 column, Agilent scientists used LC/MS/MS to identify 10-fold more proteins (approximately 400) in 4 out of 50 fractions compared to nondepleted, unfractionated crude human serum.

* Human serum data collected off-line by 2D LC/MS/MS.
** Immunodepleted with the Agilent Multiple Affinity Removal System.

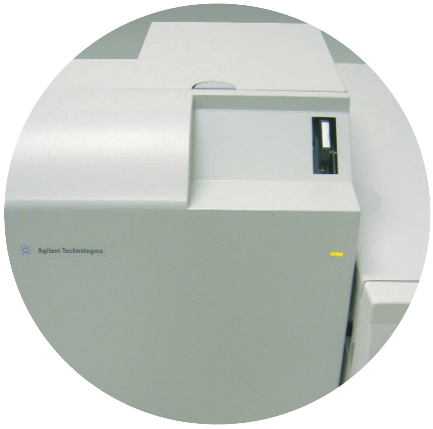


Agilent's Starter Reagent Kits for LC Columns and Spin Cartridges provide everything needed for optimal performance and easy usage of our Multiple Affinity Removal systems.

HPLC-Chip/MS and Nanoflow-LC systems for superior sample separations

No single separation technique is suitable for all protein identification studies. Some prefer the remarkable performance and ease of microfluidic chip-based separations. Others need the flexibility of nanoflow LC. That's why Agilent offers multiple separation approaches for protein identification—so every lab can find the perfect, custom-tailored fit to identify more proteins faster and with greater confidence.

The HPLC-Chip provides high resolution to increase the number of peptides found and proteins identified.



The HPLC-Chip is loaded into the HPLC-Chip Cube MS interface, which precisely positions the chip's nanospray tip orthogonal to the MS inlet to achieve maximum sensitivity and robustness.

The HPLC-Chip/MS system

Conventional nanoflow LC uses numerous intricate connections and requires substantial user skill.

For simple and high-performance analysis, Agilent offers the unique and easy-to-use HPLC-Chip.

The HPLC-Chip incorporates traditional nanoflow LC and MS components—enrichment column, analytical column, nanoelectrospray tip, and all connections between the columns and the spray tip—into a reusable, microfluidic polyimide chip the size of a microscope slide.

The HPLC-Chip's multilayered architecture dramatically reduces the possibility of leaks and clogs, and eliminates post-column dead volumes. Peak dispersion is virtually eliminated, resulting in less sample loss and narrower, better-defined peaks. The overall robustness, reliability, and ease of use are greatly improved. Most importantly, the chip ensures uncompromised chromatographic performance and delivers full separation, allowing you to identify more proteins in complex samples.

The chip integrates all electrical connections and features an embedded radio frequency (RF) tag that tracks usage and operating parameters. Information on the RF tag is automatically downloaded and updated by the system software from the HPLC-Chip Cube interface.

The HPLC-Chip Cube interface

The HPLC-Chip Cube MS interface contains everything needed to couple the HPLC-Chip to the Agilent 1100 Series HPLC system and the LC/MSD Trap XCT Ultra: the loading mechanism for chip positioning, the microvalve for nanoflow LC hydraulic connections and flow switching, and the nanospray ion source with camera for spray visualization.

In seconds, the HPLC-Chip Cube automatically loads the chip, establishing high-pressure, leak-tight fluid connections. The HPLC-Chip Cube automatically and precisely positions the chip spray tip orthogonal to the MS inlet to ensure maximum sensitivity and robustness, and makes all the necessary electrical connections and hydraulic connections to the chip.

The HPLC-Chip Cube MS interface is a standard module within the Agilent 1100 Series HPLC system and is fully integrated and controlled by the Agilent ChemStation software. Loading or replacement of the chip is simple and can be completed in a few seconds with a simple mouse click. The entire process is fully automated and requires no tools. Since it's so easy to do, each individual researcher can have his or her own dedicated chip, reducing the risk of cross contamination.

The Nanoflow-LC System for MS

If you have a diverse set of sample types or experimental objectives, you'll want to consider various separation approaches. The flexibility of Agilent's Nanoflow-LC system allows you to design a variety of separation workflows so you can apply the methods that work best in your lab and achieve exactly the separations you want. The Nanoflow-LC system can be configured for one-dimensional separation, one-dimensional separation with sample cleanup and enrichment, or full two-dimensional separation.

High-efficiency columns provide optimal separations

Agilent offers ZORBAX 300SB C18 and C8 enrichment columns (0.3 x 5 mm) for the Nanoflow-LC system. Prior enrichment improves protein detection for better results. These columns excel at concentrating and desalting low-concentration peptide mixtures, thus reducing the loading time for large-volume injections.

We offer 75 µm id, 50 or 150 mm, C18 or C8 wide-pore analytical nanocolumns featuring Agilent's patented ZORBAX StableBond (SB) technology. These reversed-phase HPLC columns are excellent for peptide separations and are extremely stable and durable. The same column is also available in a 100 µm id for applications requiring slightly higher flow rates.

For more complex digests, we also offer ion-exchange columns that can be incorporated into full two-dimensional LC separations for identifying more proteins within a sample.

Sensitivity-enhancing ultralow flows

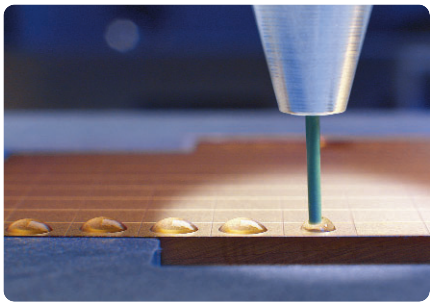
Stable, ultralow-flow rate separations are essential for highly sensitive analyses of complex protein mixtures. The Nanoflow-LC system uses cutting-edge technology to deliver ultrastable, nanoliter-per-minute flow rates and outstanding sample cleanup, concentration, and separation. Its innovative electronic flow control (EFC) system uses active feedback to provide unprecedented stability and reliability at flow rates spanning 100 nl/min to 1 µl/min. The entire system is designed to both maximize and maintain separation efficiency—switching valves, fittings, connectors, and PEEK-coated fused silica tubing have all been carefully selected to minimize dead volumes along the path flow.

Micro Collection/Spotting system for automated sample spotting

The Agilent 1100 Series Micro Collection/Spotting system is the ideal tool for single- or multidimensional chromatographic separation of complex peptide and protein mixtures for downstream analysis by either MALDI or nanospray mass spectrometry. The LC Micro Collection/Spotting system offers flexibility for multidimensional chromatography by collecting the desired fractions for subsequent analysis. This approach results in superior separations for enhanced protein identification. It also allows researchers to perform enzymatic or chemical modifications of fractions between the separation runs.

The system is designed for highly reproducible collection of small volumes at low flow rates. Depending on the application, users can choose collection capillaries with 25, 50, or 100 µm inner diameter for optimized performance. The system's Peltier cooling prevents decomposition of thermally labile compounds as well as evaporation of the microfractions. Additionally, the capability to thermostat targets for MALDI spotting promotes superior and reproducible crystallization between matrix and sample analyte.

Active-feedback flow control provides stable nanoliter flows.



The upward movement of the micro collector/spotter needle during the spotting process prevents squeezing of the droplet by the needle tip. This avoids any liquid movement along the capillary for reliable and robust LC/MALDI spotting over a wide range of droplet volumes.

Exceptional MS^n performance for protein sample analysis

The identification of low-abundance proteins requires exceptional sensitivity and specificity. However, these requirements can certainly be met in different ways. You may prefer the convenience of online integration of microfluidic nanoflow-LC and a nanospray ion source. Or you may desire the efficiency of high-throughput analyses provided by MALDI-based MS. Perhaps another particular combination of separation and MS approaches would best suit your needs. With these possibilities in mind, Agilent provides multiple approaches to help you address your most critical protein identification needs.

The HPLC-Chip Cube MS interface for performance and ease

Agilent makes using the remarkable HPLC-Chip extremely straightforward for easy protein identification with confidence. The HPLC-Chip Cube MS interface contains everything needed to couple the HPLC-Chip to the the LC/MSD Trap XCT Ultra, including the loading mechanism for chip positioning and the nanospray ion source with camera for spray visualization. The HPLC-Chip Cube automatically and precisely positions the chip spray tip orthogonal to the MS inlet to ensure maximum sensitivity and robustness, and makes all the necessary electrical connections and hydraulic connections to the chip. Use of the Agilent orthogonal dual electrode nanospray ion source reliably demonstrates favorable high signal-to-noise ratios when combined with the HPLC-Chip. And unlike other nanospray designs, it does not require needle voltage optimization or changes during the gradient.

Nanospray source for sensitivity

When used with the Agilent LC/MSD Trap XCT Ultra ion trap, the Agilent nanospray ion source provides attomole-level sensitivity for online LC separations. It is the only nanospray source to incorporate Agilent's patented orthogonal dual electrode spray technology that minimizes adjustments and keeps the source cleaner. The use of high-capacity drying gas in the system improves spectral quality, sensitivity, and reproducibility through reduction of solvent clusters and mobile phase adducts. The nanospray source is completely sealed for increased safety when working with potentially hazardous biological samples.

MALDI source for efficient, high-throughput analysis

MALDI is an ideal technique for rapid analysis of large numbers of relatively simple, well-separated samples such as peptides extracted from 2D gel spots.

Agilent's MALDI source uses pulsed dynamic focusing (PDF) to improve sensitivity. The PDF-MALDI source delivers clean, searchable spectra from only attomoles of sample. Heated drying gas directed onto the sample plate reduces the formation of matrix clusters, resulting in cleaner mass spectra. The Agilent PDF-MALDI ion source does not require tools or a vacuum environment for sample loading, ensuring fast sample processing.

Multiple samples can be spotted onto a single MALDI plate for running highly efficient MS cycles. You can target sample spots manually or, after initial positioning, the system can automatically move the sample plate for uniform sampling. In addition, the 96-well sample plates are compatible with robotic deposition. And MALDI analysis uses only a fraction of each protein preparation, so follow-up studies can be targeted to specific samples without repetitive sample preparation.

The HPLC-Chip is loaded into the HPLC-Chip Cube MS interface, which precisely positions the chip's nanospray tip orthogonal to the MS inlet to achieve maximum sensitivity and robustness.

The nanospray source provides attomole-level sensitivity with minimal adjustment and the safety advantages of a sealed source.

The PDF-MALDI source provides fast, easy analysis and outstanding sensitivity for peptide digests.

LC/MSD Trap XCT Ultra ion trap MS

The LC/MSD Trap XCT Ultra consistently matches or exceeds the sensitivity and scan speed of other three-dimensional ion traps and two-dimensional, linear ion traps. For optimal sensitivity, the XCT Ultra combines a multipole design that increases trap capacity and a Smart Target function that maximizes trap capacity usage. At the same time, it retains the resolution and scan speed advantages inherent in a true three-dimensional ion trap.

Faster scanning helps identify more peptides

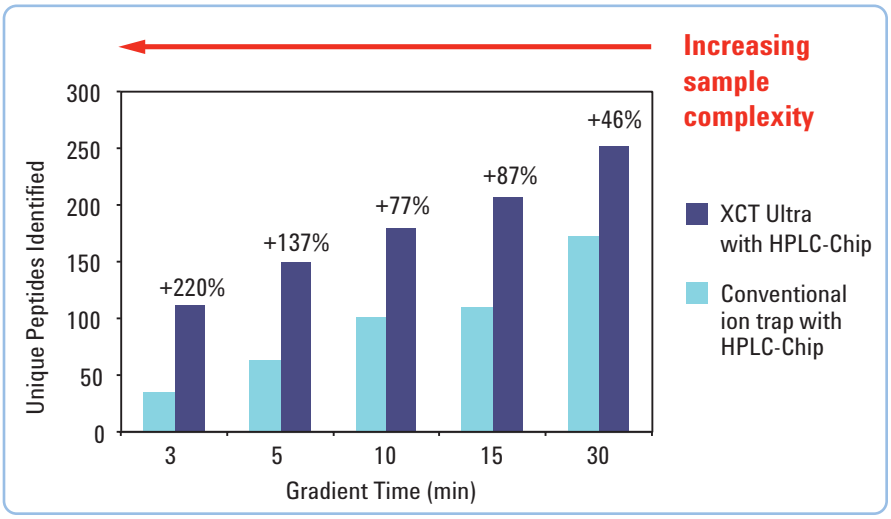
The XCT Ultra redefines ion trap performance by implementing a high-performance data acquisition subsystem that acquires up to three times as many scans at a given scan speed due to dramatically shortened scan cycles. Acquiring more scans—and therefore more spectra—over a chromatographic peak helps you identify more lower-abundance peptides in the presence of higher-abundance coeluting peptides. This feature also improves quantitative accuracy, which can aid in the discovery and validation of biomarkers and diagnostic markers.

Advanced capabilities for easier, faster identifications

The LC/MSD Trap XCT Ultra has an arsenal of sophisticated data acquisition features that allow you to rapidly acquire multiple levels of the most informative and relevant data from each scan.

- Static and active data acquisition features perform up to 5 stages (MS^5) of fully automated, data-dependent MS/MS in a single run.
- Our unique SmartFrag collision-energy ramping and adjustable fragmentation width (AFW) ensure optimum fragmentation for greater product ion generation and structural information using fewer stages of MS/MS.
- Maximum resolution scans over a narrow mass window allow more accurate +1, +2, and +3 charge state determination of peptide ions in both MS and MS/MS analyses.
- A higher-resolution enhanced peptide scan mode yields extremely high-quality data from more unique ions—even within complex digests.
- A powerful data-dependent neutral-loss mode for identifying phosphopeptides
- Simplified results navigation—data from each run is stored in a single file. A familiar, hierarchical tree structure and special filters allow easy navigation and straightforward identification and extraction of precisely the data or data subset you need.
- Visual Basic-driven, user-designed automation through custom scripts for data analysis and reporting. A wide range of MS-specific commands have been built-in.

The LC/MSD Trap XCT Ultra has an outstanding combination of resolution and scan speed so that you can obtain high-quality results in minimal time.



The ultrafast scanning of the LC/MSD Trap XCT Ultra increases the number of peptides identified. The more complex the sample—which was simulated by shortening the gradient time—the greater the benefit of the fast scanning.

Powerful software tools identify more proteins quickly

High quality data is necessary, but not sufficient. It takes the right software tools to turn good data into useful, relevant information. Agilent offers the Spectrum Mill MS proteomics workbench, a comprehensive suite of software tools that turn your MS^n data into answers.

The Spectrum Mill workbench provides you with:

- Intelligent spectral extraction and quality assessment
- Database searching and *de novo* sequencing
- Automated and interactive results validation
- Quantitative and semi-quantitative analyses
- Cross-experiment result summaries
- Multiple options including MS/MS search for MS/MS spectra and peptide mass fingerprinting (PMF) search for MS spectra

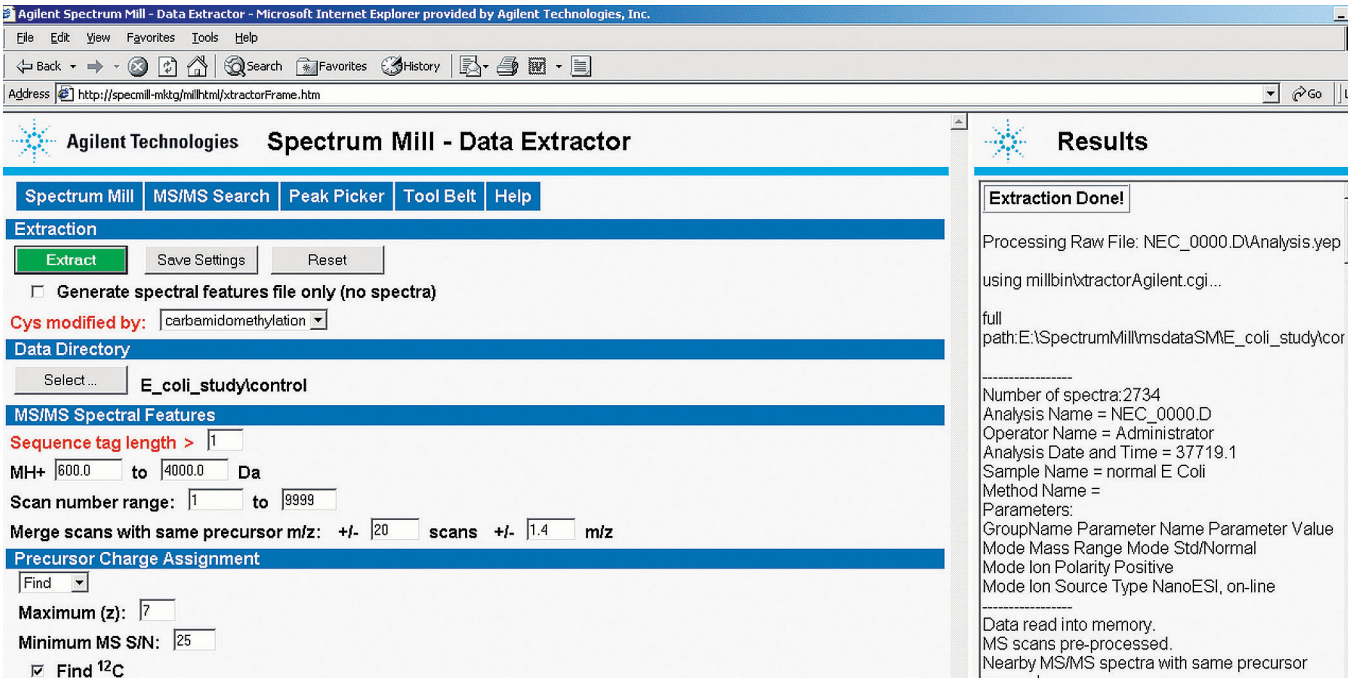
Intelligent spectral extraction speeds protein identification

The extreme speed and efficiency with which our Spectrum Mill workbench searches protein databases is due in large part to its intelligent extraction and processing of mass spectra. By identifying and excluding noise and poor quality spectra prior to searching, the search speed is greatly increased.

Additionally, our innovative MS/MS database search algorithm uses refined parallelization to rapidly process data—without the creation of gigantic index files. You can use the identity mode to find unmodified peptides, or the variable homology modes to search for mutations and modifications.

Automatic and manual match validation

The Spectrum Mill workbench will automatically validate matches for you based on overall score and percent-scored peak intensity. You can interactively review proposed matches and compare them to actual MS/MS spectra. In addition, the adaptive search algorithm enables unvalidated spectra to be re-searched using alternate parameters or databases.



The speed and efficiency with which the Spectrum Mill workbench searches protein databases is due in large part to intelligent data extraction and data quality assessment before searching begins. The Spectrum Mill extraction software assesses MS/MS spectral quality based on sequence tag length and signal-to noise criteria.

Quantitative as well as qualitative information

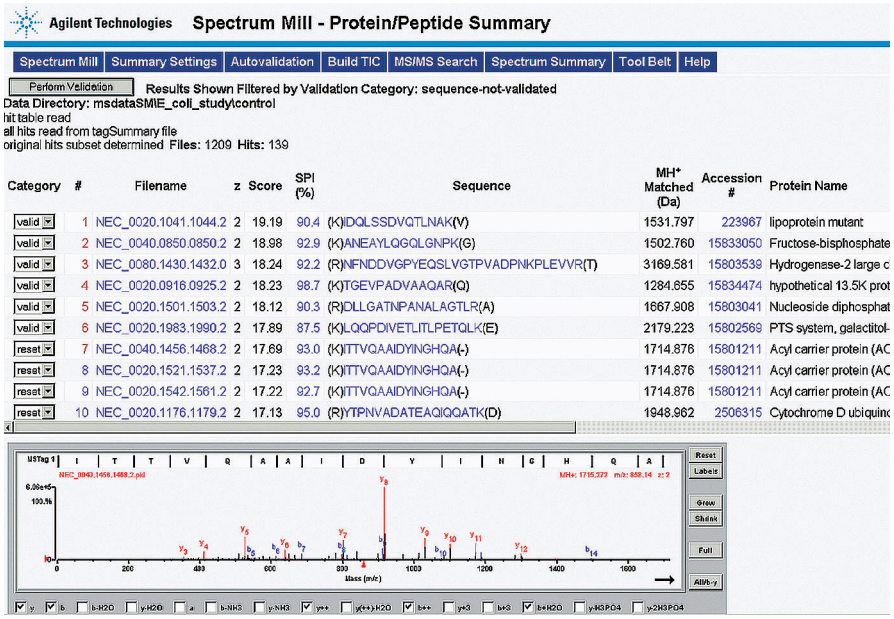
The Spectrum Mill workbench can compare relative protein abundances based on mean peak intensities of all the component peptides from a protein; this method can reveal two- to five-fold changes in relative abundances. When you need more precise quantitation, the Spectrum Mill workbench fully supports stable isotope and other labeling experiments.

De novo spectral interpretation for peptides not found in any database

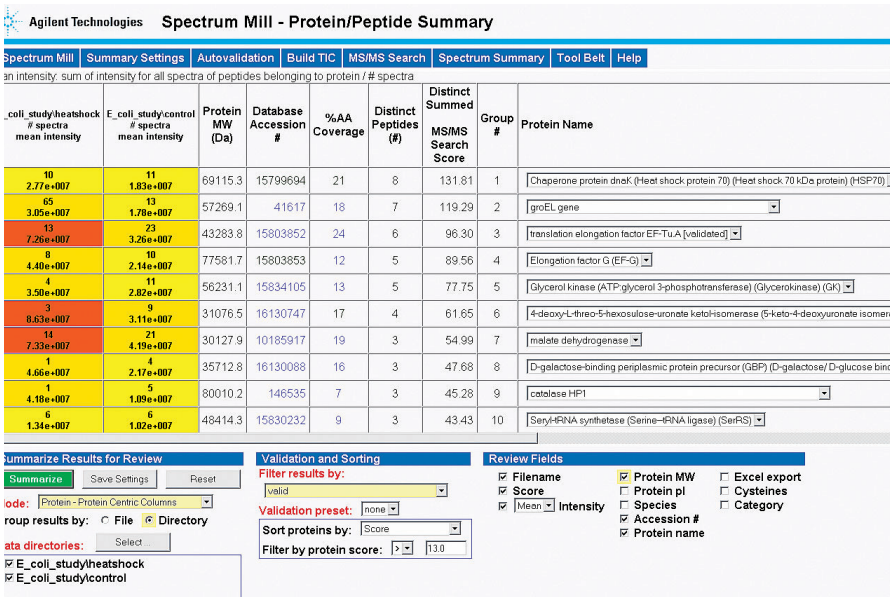
The *de novo* sequencing algorithm uses advanced graph theory to generate a ranked list of potential peptide sequences. It discards unrealistic solutions and compensates for common spectral difficulties such as noise and incomplete fragmentation.

Complex data made accessible

The Spectrum Mill workbench summarizes and correlates results in ways that provide insight to answers to biological questions. Tables of results can include protein identities, percent coverages, and relative abundances. You can compare large data sets across multiple experiments and summarize the results at the protein level.



Interactive validation facilitates comparison of the proposed match to the actual MS/MS spectra.

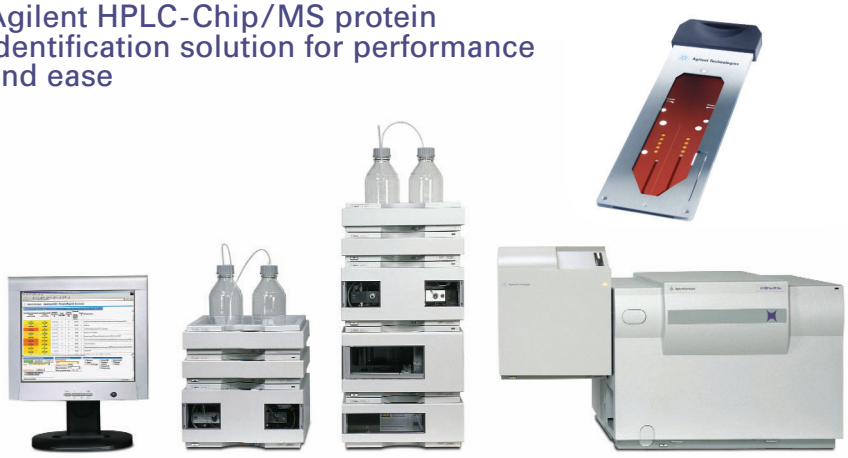


Protein-protein mode allows comparison of data, including color-coded relative abundance information, from multiple samples.

Protein identification solutions from Agilent

Our protein identification solutions combine Agilent's advanced tools for protein analysis into integrated solutions for analyzing complex mixtures of proteolytically digested proteins. Whether you require convenient, flexible, or high-throughput analyses, we provide a direct solution that delivers high performance.

Agilent HPLC-Chip/MS protein identification solution for performance and ease

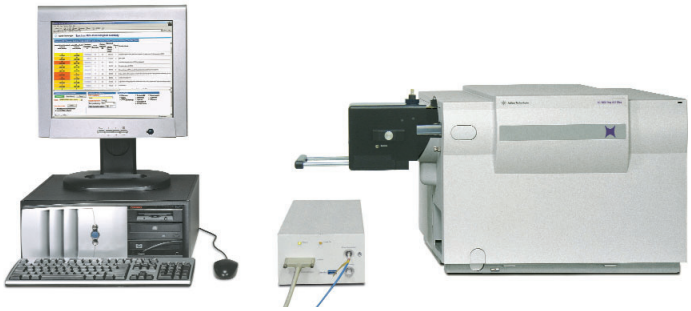


Identify more proteins more quickly and with greater confidence. The HPLC-Chip's multilayered microfluidic architecture has fewer components and reduced flow path length for less sample loss, minimal clogs or leaks, and sharper peaks. Sample enrichment, fast loading, high-resolution separation, and direct electrospray into our XCT Ultra ion trap system are all seamlessly integrated into a single, reusable polymer chip. Experience high-level chromatographic performance when post-column dead volume is eliminated.

Agilent Nanoflow proteomics solution for flexibility

The Agilent Nanoflow Proteomics Solution carefully integrates state-of-the art nanoflow LC and ion trap MS^n to create a flexible solution for the separation and identification of low-abundance proteins. The system provides a wide range of separation choices that you can adapt for your sample and experimental objectives.

Agilent MALDI proteomics solution for high-throughput analysis



When working with samples such as tryptic digests of two-dimensional gel spots, the LC/MSD Trap XCT Ultra and MALDI source offer a very fast way to analyze large numbers of samples. Multiple samples can be spotted onto a single MALDI plate for efficient MS run cycles. Agilent's MALDI source uses pulsed dynamic focusing (PDF) to improve sensitivity. The PDF-MALDI source delivers clean, searchable spectra from only attomoles of sample.

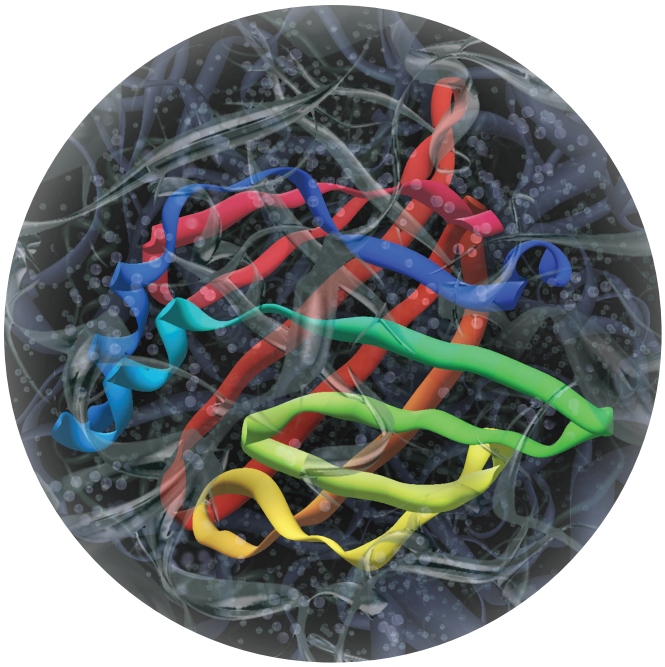
Protein ID solution products	HPLC-Chip/MS	Nanoflow LC	PDF-MALDI MS
Spin Filters	Recommended	Recommended	Recommended
Multiple Affinity Removal Systems*	Recommended	Recommended	Recommended
mRP-C18	Recommended	Recommended	Recommended
Spin Concentrators	Recommended	Recommended	Recommended
Protein In-gel Tryptic Digestion kit	Recommended	Recommended	Recommended
Peptide Cleanup C18 spin tubes and pipette tips	Recommended	Recommended	Optional
MALDI-MS Matrices	N/A	N/A	Standard
Peptide and Protein standards	Optional	Optional	Recommended
HPLC-Chip	Standard	N/A	N/A
HPLC-Chip Cube MS interface	Standard	N/A	N/A
Agilent 1100 Series Nanoflow LC System for MS	Standard	Standard	Optional
Autosampler cooling	Recommended	Recommended	Optional
Agilent orthogonal nanospray source	N/A	Standard	N/A
Agilent PDF-MALDI source	N/A	N/A	Standard
Agilent LC/MSD Trap XCT Ultra	Standard	Standard	Standard
Micro fraction collector/plate spotter	Optional	Optional	Optional
Spectrum Mill MS Proteomics Workbench	Recommended	Recommended	Recommended
Protocols for sample preparation and MS analysis	Standard	Standard	Standard
Familiarization/troubleshooting exercise	Standard	Standard	Standard
Additional training and support	Standard	Standard	Standard

N/A = not applicable

* Removal systems optimized for either human/monkey or mouse/rat serum samples. Can be applied to other mammalian serum samples.

About Agilent's Integrated Biology Solutions

Agilent Technologies is a leading supplier of life science research systems that enable scientists to understand complex biological processes, determine disease mechanisms and speed drug discovery. Engineered for sensitivity, reproducibility and workflow productivity, Agilent's integrated biology solutions include instrumentation, microfluidics, software, microarrays, consumables and services for genomics, proteomics and metabolomics applications.



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